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INDUCTOR AT DIRECT OVARIAN ACTION, *THE CHORIONIC GONADOTROPIN* IN ARTIFICIAL REPRODUCTION OF AFRICAN CAT FISH (*Clarias Gariepinus*, BURCHELL 1822)

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ABSTRACT

Twenty four (24) *Clarias gariepinus* broodstock, from the fish farm Zahra of Rouani at Hassi El-Fhal (Ghardaia, Algeria), divided into groups of 6 males and 6 females, received respectively "chorionic gonadotropin hormone" from pregnant woman (HCG SR) and commercial HCG (HCG CO).

The two batches have responded favourably, the numbers of eggs obtained were 84.16 ± 50.73 g under HCG SR and 119.333 ± 15.319 g under the HCG CO. The amounts of milt were 5.167 ± 0.753 ml under the HCG SR and 5.333 ± 0.816 ml under HCG CO.

The hatching rates were comparable, and were 68.16 ± 22.04% under the HCG SR and 81.66 ± 1.21% in HCG CO.

Our trials are encouraging and plead in favour of development of such protocol in order to replace non purified pituitary glands whose cost price is higher, by serum β HCG, a specific hormone capable to stimulate the ovule in dormant phase.

KEYWORDS: *Clarias gariepinus*, Reproduction, HCG, Pituitaries Glands Gametes, Hatching

INTRODUCTION

In Algeria, aquaculture development by introduction of *Cyprinidae*, *Percidae*, *Siluridae* and *Clariidae* requires a technical expertise of production. Among these species, the *Clarias gariepinus* (Clariidae) is a choice model of fish farming because of its adaptation in the South of Algeria, mainly by its rapid growth, its resistance to pathologies, to variability of its diet and to its reproductive capacity in confined spaces (Janssen. 1986). In Algeria exploitation of this specie in fish farm has been limited by lack of alevins due to non technical expertise of natural and artificial reproduction. In fish farming, production of *Clarias gariepinus* requires the control of the reproduction process to qualitatively and quantitatively optimize spawnings products (Pouomogne *et al.*, 1998; Brummett, 2007). To this end, use of hormonal factors is necessary to require technique perfectly prepared repeatable and economically accessible. This work consists in

using on African cat fish *Clarias gariepinus* (Bruchell, 1822) an inductor at direct ovarian action the serum β HCG from pregnant women due to its weak cost compared particularly to pituitary. Placental hormone of mammal, the human chorionic gonadotropin (HCG) according to Pickford and Atz (1957) has a stimulating effect on gonads of both fish sexes: this is due to its high content in FSH and LH (Shahani and Rao 1964). For this, different doses of serum β HCG from pregnant woman and commercial β HCG have been used on spawners of *C. Gariepinus.* Effect evaluation of these two hormones is expressed by quantity of milt and eggs obtained and so of hatching rate of fertilized eggs.

MATERIAL AND METHODS

Experimentation on hormonal induction by SR HCG and CO, HCG was performed in 2013 in fish farm Zahra at Hassi El-Fhal (Ghardaia – Algeria).

Preparation of Serum Hcg (Hcg Sr)

The serum taken out from pregnant women, in laboratory of Dr Zibouche A (Ain-Defla – Algeria) was characterized by HCG rate upper to 1500UIm/ml. The concentration determination of HCG (entire molecule and free beta sub-units) has been performed by the dosage method of Beckman Access Total β HCG using an immuno-enzymatic technique luminescence-chemical (CMIA) with magnetic particles for dosage of total β HCG in human serum, with help of Immuno-analysis System Access. The serum samples containing HCG were packaged in small flasks of 02ml and transported at +4°c towards experimental station Zahra (Ghardaia, Algeria).

Choice of Spawners and Hormonal Induction

Spawners of *Clarias gariepinus* were from fish farm production of extensive type practiced in the farm of Rouani M. Situated in Hassi El-fhal, wilaya of Ghardaia (Algeria). Hormonal induction has been tested on males and females spawners with variables weight whose characteristics are given on table 1. Commercial HCG (HCG CO) was administered on the base of 4000UI/ kg of live weight (Legendre and al 1991). However, the dose of serum β HCG from pregnant women (HCG SR) has been raised to 5000 UI/kg to compensate impurities present in serum.

Table 1: Weight of Spawners of C. Gariepinus (A to F) and Hormonal Doses (SR HCG and CO HCG) Administered M: Average Weight of Eggs (G)

Hormones	Sexe / Dose		Dose Administered / N of Spawners					
		A	B	C	D	E	F	M
HCG SR	*C. gariepinus* (g), Female	990	800	875	1800	1080	1100	1107.50 ± 358.55
	Dose (UI/kg)	4950	4000	4375	9000	5400	5500	
HCG CO	*C. gariepinus* (g), Female	1200	1090	825	950	1010	1150	1037.50 ± 138.12
	Dose (UI/kg)	4800	4350	3300	3800	4040	4600	
HCG SR	Males (g)	810	950	1100	1040	1030	980	985 ± 100.15
	Dose (UI/kg)	4050	4750	5500	5200	5150	4900	
HCG CO	Males (g)	920	960	1020	1040	970	990	983.33 ± 43.20
	Dose (UI/kg)	3680	3840	4080	4160	3880	3960	

Reproduction

After selection and weighting, spawners were anesthetised and simulated by doses of HCG SR and by HCG CO specifically to each spawner (Tab 1).

Stripping necessitated a thermal accumulation of 275°Hours (Richter *et al.*, 1987; Ducarne and Micha, 2003); for 10 hours at 28°C, specific temperature of the water at the Rouani Farm. Eggs of *C. gariepinus* obtained were weighted then, fertilized by milt of sacrificed males (De Graaf and Jansen 1996). This laitance was quantified by the volumetric method. Eggs incubation of each female was made on spawning into aerated bins of 1000 l.

Hatching rate was estimated by counting unhatched eggs according to the eggs mass obtained from each spawner. Counting of viable larvae confirmed hatching rates.

Data Evaluation and Treatment

Efficiency evaluation of HCB SR and HCG CO induction in spawners of *C. Gariepinus* was made by egg mass quantification obtained by stripping of each female and to milt's quantity produced by males and so by hatching rate of fertilized eggs.

Parameterised or non-parameterised hypothesis tests have been applied to validate the results of the reproduction. Two software have been used in statistical calculations and developing of regression lines: the Excel Stat version 2009 and R (R Development Core Team 2010).

Results

The results relate to the description of the lots *Clarias gariepinus* and homogeneity compared to the experience as well as on reproduction where the quantitative aspect is dealt with only taking into account the quantities of gametes (eggs and milt) produced under the effect of HCG and HCG SR CO. However, qualitative aspect was expressed only by hatching rates of eggs obtained under action of HCG SR and HCG CO.

Description of *Clarias Gariepinus'* Spawners

Lots of *Clarias gariepinus*, spawners, used in evidencing activity of serum HCG (HCG SR) and synthetic HCG (HCG CO), were homogeneous (females and males. t. test, $p \geq 0.66$). Average weight of spawners (Tab 1, fig 1) females having received HCG SR was 1107.50 ±358.55g and varied from 800 to 1800 g. Celui des males est de 985±100.150 g et varie de 810 à 1100 g. Pour les géniteurs (Tab. 1, Fig. 1) femelles ayant reçu l'HCG CO, le poids est de 1037.50±138,12 g et varie de 825 à 1200 g, alors que celui des males est de 983.33±43.20 g et varie de 920 à 1040 g.

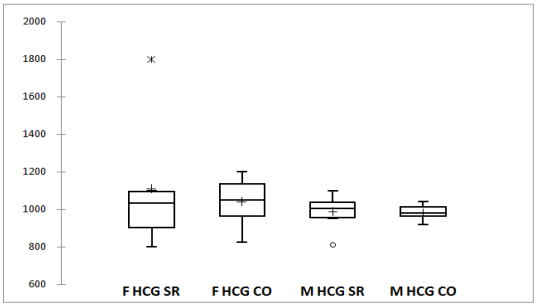

Figure 1: Weight Variability (G) of *C. Gariepinus* Males And Females Spawners. *F: Females, M: Males, (*): Outlier*

That of males was 985±100.150 g and varied from 810 à 1100 g. For spawning females (Tab 1. Fig 1) having received HCG CO, the weight was of 1037.50±138.12 g and varied from 825 to 1200 g, while that of males, was 983.33 ± 43.20 g and ranged from 920 to 1040 g.

Reproduction

Quantitative aspect of the reproduction, in which only eggs quantities and milt produced by spawners of *C. Gariepinus* were taken into account (Fig 2, Tab 2), allowed us to note, that there was no significantly difference between females (Fig 2a) having received HCG SR and those ones induced by HCG CO (t. test, p=0,13).

Table 2: Quantities of Eggs and Milt Produced by C. Gariepinus (A to F) under Effect of SR HCG and CO HCG. M: Everage Weight of Eggs /Kg of Live Weight

Hormones/ Sexe	Gametes	Quantities of Gametes / N of Spawner						
		A	***B***	***C***	***D***	***E***	***F***	**M**
HCG SR/ Female	Eggs (g)	5	60	80	155	115	90	76.00
HCG CO/ Female	Eggs (g)	120	140	130	96	110	120	115.02
HCG SR/ Males	Milt (ml)	5	6	5	4	5	6	5.25
HCG CO/ Males	Milt (ml)	5	6	6	6	4	5	5.43

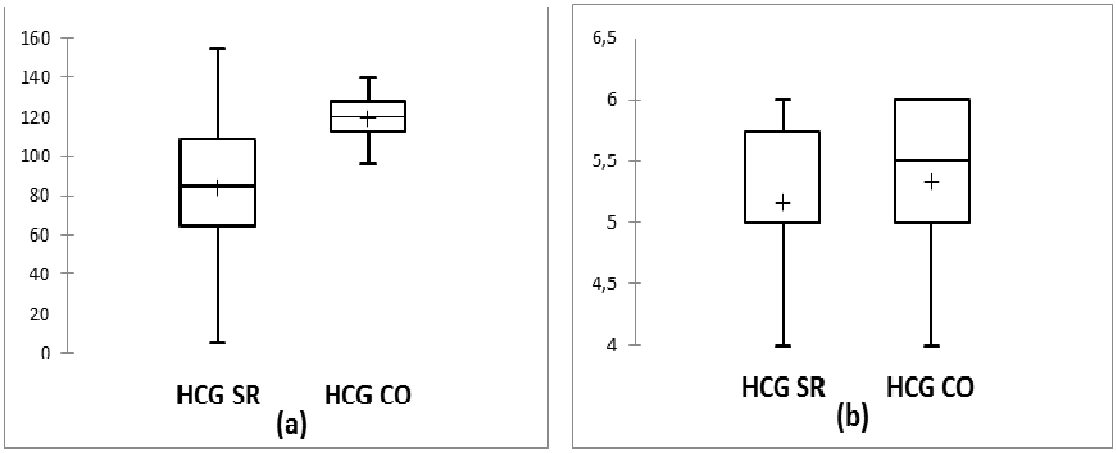

Figure 2: Variability of Eggs Quantities in G (A) and Milt in Ml (B) Produced By *C. Gariepinus*, Under Action of HCG SR and HCG CO

This acknowledgment was also valuable to males (Fig 2b) which have received these same hormones (t. test, p=0, 72).

The average amounts of eggs produced (Fig. 2a, Tab. 2) under the action of HCG SR were 84.167 ± 50.736 g and ranged from 5 to 155g , while those produced by the action of HCG CO were 119.333 ± 15.319 g and varied from 96 to 140 g.

The average amounts of milt produced (Fig. 2b, Tab. 2) under the action of HCG SR were 5.167 ± 0.753 ml and ranged from 4 to 6 ml. Those produced under the action of HCG CO were 5.333 ± 0.816 ml and also ranged from 4 to 6 ml.

From a qualitative point of view, there was no significant difference (t. Test, p = 0.16) between the rates of hatching eggs from induction by HCG and HCG SR CO (Tab. 3, Fig. 3).

Table 3: Eggs Hatching Rates of *C. Gariepinus* Obtained by Induction of HCG SR and by HCG CO

Hormone/ Sex	Spawners / Hatching Rates (%)					
	A	B	C	D	E	F
SR HCG / Female	26	66	72	88	82	75
CO/ HCG Female	81	83	83	80	81	82

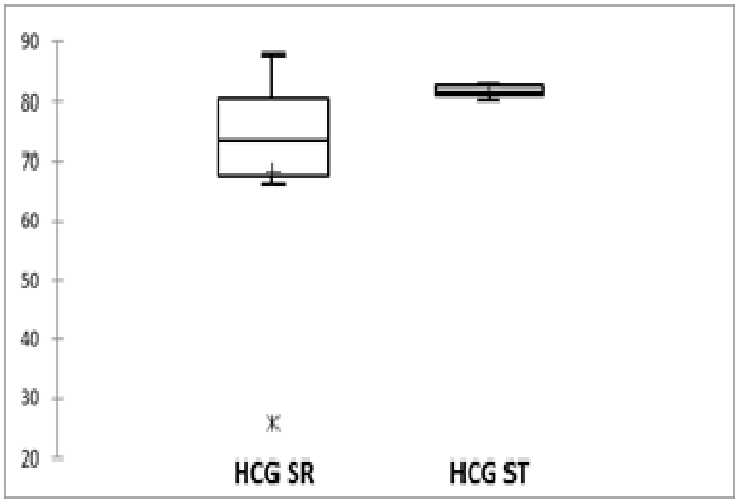

Figure 3: Variability of Eggs Hatching Rates (%) Obtained by Induction of *C. Gariepinus* of HCG SR and. HCG CO; *(*): Outlier*

Means hatching rate (Fig. 3) were 68.16 ± 22.04% in HCG SR with a variation from 26 to 88% and 81.66 ± 1.21% in HCG CO with a variation 80 to 83%.

This wide variation outbreaks obtained through HCG SR (26-88%) was mainly due to the individual A which was also apparent in the form of outlier in Figure 3.

DISCUSSIONS

Spawners which were subject of hormonal stimulation based of HCG SR and HCG CO were homogeneous and had a weight variation which did not influence in any way the qualitative and quantitative aspect of reproduction.

The spawning of this species reached sexual maturity at the age of 5 to 10 months, reaching a weight of 200-500 grams (Richter *et al*., 1982; De Graaf and Janssen, 1996; Çek and Yilmaz, 2007).

Nevertheless females weighing more than 200g, as were the case of the parents that we have induced, accused better fertility (Legendre 1992).

It is also known that fertility of the major part of teleostei linearly increased with females' weight (Bagenal 1978; Legendre, 1986 and Legendre and Ecoutin 1989).

Çek and Yilmaz (2007) showed that maturity is much more related to the size that has age. To this must be added the importance of seasonality that affects spawning of *C. gariepinus* (Brzuska, 2003). It is important to note that this spawning period may be extended by maintaining a constant temperature, photoperiod and diet (Richter *et al*., 1995; Viveiros *et al*., 2002).

Average eggs quantities obtained under HCG SR and HCB CO, respectfully 76g/kg and 115g/kg (Tab.1 and 2), were comparable to those obtained in Cameroun by induction of GNRHa, and whose average weight was 77 g/kg (Cacot 2006). The work of Brzuska (2004) has found that the amounts of obtained eggs and their outbreaks were higher with Ovopel containing an analogue of GnRHa with the carp pituitary. Milt quantities obtained were sufficient to fertilize eggs coming from reproduction: 1ml of milt allowed a fertility of 50 kg of eggs (Hogendoorn and Visman, 1980). Eggs hatching rates obtained under HCG SR were comparable to the ones obtained with HCG CO which were respectively of 68.16±22.04% and of 81.66±1,21%.

These results were equal to those obtained in standard conditions at the station of Koupa-Matapit in Cameroun which were of 82.3% (Cacot, 2006). Between 25°c and 29°c, hatching rates were upper to 75% (Legendre 1992); which confirm our results obtained at a temperature of 28°c. The weak eggs hatching rate coming from individual A under HCG SR (Tab 3) represented by outlier of figure 3 which influenced the average of hatching, had no relation with experience conditions and was linked to intrinsic factors of individual itself which may had an impact on eggs quality (Nguenga *et al.*, 2000). In this sense we consider that this has no connection with the quality of the egg fertilization which was considered homogeneous since the correlation coefficients (R2) found between the amounts of obtained eggs and hatching rate eggs were in the range of 0.88 with HCG SR and 0.87 with HCG CO (Fig. 4 a, b).

In view of similar conditions with which fertilization was performed and so the homogeneity of spawner's weight, eggs quality of individual A, under HCG SR, was probably due to others physiological factors linked to oogenesis.

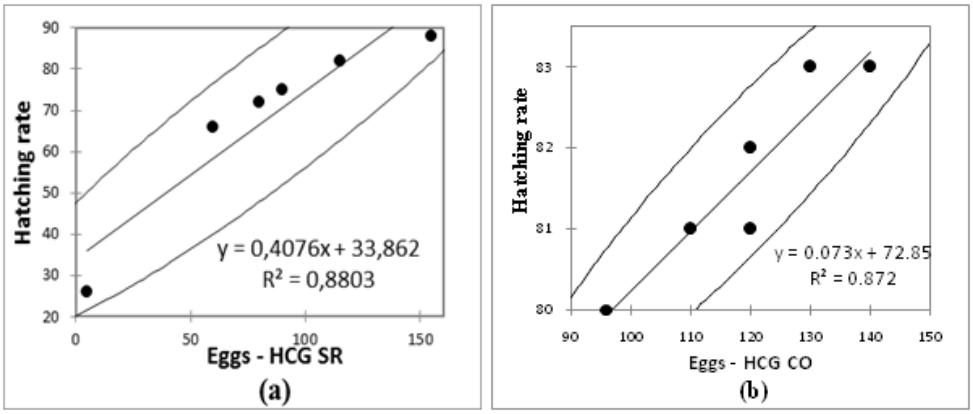

Figure 4: Correlation between Hatching Rates and Eggs Quantities Obtained Under HCG SR (A) and HCG CO (B)

CONCLUSIONS

This experience performed at Zahra farm in order to compare two hormonal inductors, the Serum β HCG of pregnant women (HCG SR) and the commercial β HCG (HCG CO) on spawners of *Clarias gariepinus* has allowed us to note that eggs quantities produced and hatching rates are comparable. Our trials are encouraging and plead in favour of development of such protocol to replace non -purified pituitary glands and whose the cost price is very high by the Serum β HCG a specific hormone capable to stimulate ovule in dormant phase. HCG CO may be substituted by HCG SR. That may open new opportunities of ovarian stimulation with *Clarias gariepinus*.

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